BOTANY HONOURS PROJECT

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POLLEN MORPHOLOGY IN RELATION TO POLLINATION SYNDROMES AND SECTIONS IN *ERICA*

ABSTRACT:

Phylogenetic studies are often aided by the use of palynological characters. Alternatively variation in the characters are interpreted as an aptation to a particular vector. Forty *Erica* species were selected on which to conduct a palynological study to determine the reason for any variation in characters which may be observed. Species were selected to represent the three putative pollination syndromes associated with this genus. These species were also from 23 sections. LM and SEM was used to collect data concerning pollen characters. These included: aperture shape, number, position and margin, exine sculpturing (ornamentation) and - stratification, tetrad shape and size. Variation did not occur in all these characters. However aperture margins, exine sculpturing and tetrad shape and size did show alternative states. These variations occurred randomly across the entire spectrum of species studied. Preliminary results therefore indicate that variation cannot be ascribed to the type of pollinator involved and similarly these characters do not substantiate the current classification at subgeneric and sectional levels. It is suggested that more species from each section be analyzed and that TEM be used to reveal exine stratification clearly before the latter half of the previous statement can be made with certainty. Identification to species level on the basis of exine sculpturing is possible.

INTRODUCTION:

Palynology is the study of pollen and spores. The term, introduced by Hyde and Williams in 1945 (Erdtman, 1971), is derived from Greek meaning to strew or sprinkle (possibly referring to fine meal) and relates to the Latin word "pollen" which means flour or dust (Traverse,1988). Palynology deals chiefly with pollen grain walls and not the live interior (Erdtman,1971). Consequently the morphological aspects of the exine are important. Due to the size of pollen grains (measured in microns) microscopy plays a major role in this study field. The application of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) has allowed many new data to be obtained and consequently led to improved interpretation of many pollen grain characters.

Palynology has therefore become increasingly important in terms of its contribution to angiosperm systematics and phylogeny. It has been particularly useful at higher taxonomic levels as shown by the proposed phylogeny of Poales and Restionales by Linder and Ferguson (1985). A group of pollen characters which are phylogenetically useful are generally studied, and these characters are used to substantiate or refute other systems of angiosperm classification. Furthermore these characters are used to outline certain phylogenetic trends observed in different

groups of angiosperms. Walker and Doyle (1975) for instance show that the most primitive subclass of dicotyledons in the Cronquist classification system, the Magnoliidae, is supported in this position by having the most primitive type of angiosperm pollen (monosulcate and monosulcate-derived pollen). The non-magnoliid Dicotyledoneae exclusively possess tricolpate and tricolpate-derived pollen: an apomorphic aperture shape. Within this group the Dilleniidae, which includes the family Ericaceae, has tricolporate pollen as opposed to the more plesiomorphic tricolpate pollen of Ranunculiales.

Not only is the aperture shape of phylogenetic-evolutionary significance, but aperture position, number and structure are also of importance. Further pollen characters which are phylogenetically useful are pollen wall architecture (which includes wall stratification, exine structure and -sculpturing), pollen unit (whether shed in monads or tetrads), pollen grain polarity, symmetry shape and size. Each one of these characters can be described in terms of their evolutionary development and the most plesiomorphic and apomorphic states identified (Walker and Doyle, 1975).

Although data obtained from ultrastructural features are mostly of value at higher taxonomic levels, Stuessy (1990) states that there are pollen grains useful at all hierarchical levels due to a variety of selective forces

which may cause variation acting on these grains. Some taxa may show little variation (stenopalynous) whilst others vary greatly (eurypalynous). Differences may however be subtle and on closer inspection reveal features which are useful at generic subgeneric and sectional level (Steussy 1990).

Use of palynology in Erica

As Guthrie and Bolus (cited by Baker and Oliver, 1967) emphasized, the classification of the largest genus in the Cape flora (Oliver, Linder and Rourke, 1983), poses very difficult problems. Due to the relative divergence of the floral characters, the general structuring of the genus into subgenera and sections is based on these characters. No comprehensive palynological study has been attempted in *Erica* and any such data may be useful in determining phylogenetic relationships at these low levels of classification.

Those species occurring in the Western Cape have been categorized into pollination syndromes (Rebelo, Siegfried and Oliver, 1985). This has been done according to floral characters as they pertain to particular pollinators as described by Faegri and Van der Pijl (1979) and observations by Rebelo et al (1985). However, it has been suggested that the morphological variations noted in pollen grains reflect certain aptations which facilitate transport by a particular vector (Ferguson and Skvarla, 1982; Crane, 1986; Punt,

1986). Variations in palynological characters could therefore be interpreted as being a consequence of either one of the above parameters.

For a meaningful interpretation of the results the classification of *Erica* as it is currently accepted, the basis on which the division into pollination syndromes is done, the structural-functional relationship of pollen grains and how the exine may be affected by the type of pollinator should be addressed.

Classification of *Erica*

The first *Erica* which can be positively identified as having come from the Cape is mentioned by Plukenet in "Amalgesti Mantissa Botani", published in 1700. Between then and 1967 ca. 605 *Erica* species have been described south of the Limpopo River (Baker and Oliver, 1967). Most classificatory work on these ericaceous plants was done by Guthrie and Bolus who dealt with and classified 469 species. In 1965 a revision of the whole genus by Dulfer was published (Baker and Oliver, 1967).

Members of the family Ericaceae ~~is~~ are characterized by sympetalous corollas, bilocular anthers opening by pores, a superior ovary and stamens mostly twice the number of corolla lobes. Further diagnostic features of southern African members are small narrow leaves and anthers having

apical pores. Klotzch in 1834 was the first to classify the family into three tribes (Oliver, 1989). These were recognized by the degree of fusion between stamens. Bentham (1876) introduced subtribes which were later included into Drude's (1897) classification. Drude however used classificatory terminology still recognized today i.e. the use of the terms subfamily and tribes; i.e. tribes were termed subfamilies and subtribes were termed tribes. Three subfamilies are recognized viz. Arbutoideae, Ericoideae and Rhododendroideae of which only Ericoideae is represented in Africa (Baker and Oliver, 1967). Watson et al (cited by Oliver, 1989) suggested a new system of tribe classification but the classification into tribes currently most accepted is that of Stevens (1971). The subfamily Ericoideae is divided into three tribes: Calluneae, Ericaceae and Salixidae. Both Ericaceae and Salixidae are found in southern Africa and are separated from Callunaea in that they have non-saggitate leaves and Ø-2 bracteoles. The distinction between the remaining two is that genera in Ericaceae have dehiscent capsular fruit, those in Salixideae a dehiscent or indehiscent drupe or berry (Oliver, 1989).

Calluneae is monotypic. Ericaceae has five genera and the remaining 18 fall in Salixidae. These 24 genera are classified on the basis of ovary cell numbers and ovules per ovary coupled with number of corolla, calyx and stamen parts (Table 1).

Table 1 Example of ~~how~~ which characters are used to distinguish genera

Genus	corolla lobes	calyx lobes	stamens	ovary cells	ovules/ cell
Erica	4	4	8	4	many
Anserica	2	4	4	2	1
Sympieza	2	2	4	2	1
Scyphogyne	4	4	4	1	1

All genera excluding Erica are collectively known as the Minor Genera of the Ericoideae as these genera include relatively low numbers of species compared to the ca. 670 species (Oliver, 1989) found in *Erica*.

Erica is thus delimited by having the flora formula B1, br2, K4, C(4), A8, G(4), S ∞. Oliver (1989) states that most of the variation within the genus is related to inflorescence structure, size, shape and colour of the calyx and corolla, and shape of the awns on the anthers. However a study of the subfamily Ericoideae (Oliver, 1989) has shown variation within and polyphyletic origin of the Minor Genera which makes their recognition as distinct entities highly tenuous. Oliver further states that the tribes Ericaceae and Salixidae need to be recircumscribed and genera will therefore need to be reallocated. One large genus *Erica* may be the end result.

For reasons of making the large genus more manageable, Guthrie and Bolus in Flora Capensis of 1905 delimited five

subgenera and 41 sections which are still recognized presently. The subgenera are classified according to the gross morphology of the corolla or corolla and sepals. Within each subgenus the sections are delimited by whether flowers are borne in an axillary or terminal position, anthers are exerted or included and also by more detailed character description of the variation within the shape and size of the corolla.

Division according to pollination syndromes

The categorization of the 426 *Erica* species into pollination syndromes is done primarily on the shape of the flowers (Rebelo et al 1985). Delimitation is not exclusive but follows a general trend and is also based on extensive observations in the field by Rebelo et al (1985). Three pollination syndromes are recognized which are ornithophily, entomophily and anemophily.

The flower shapes of bird-pollinated species are long and tubular, being either straight or curved. Orifices are wide enough (> 2mm) to allow beak penetration by sunbirds (Nectariniidae). All ornithophilous species fall within the subgenus Syringodea.

The most distinguishing feature characterizing entomophilous species is the elaborate appendages borne on the anthers. Baker and Oliver (1967) state that the appendages position

the anthers in the centre of the corolla to maximally facilitate pollen deposition on visiting insects. The majority (80%) of Erica species are insect pollinated and are represented in all five subgenera. A diverse range of insects visit *Ericas*, but assigning any particular flower shape to a specific pollinator is difficult as there are many intermediate shapes and insects tend to visit non-selectively (Rebelo et al, 1985). However a distinction can be made according to whether species are exclusively visited by long probocid flies or not. The former differ in shape from bird pollinated species in having a small orifice (< 2mm) with large, spreading star-shaped corolla lobes. Long probocid flies are not selective and visit other non-open mouthed species (viz. globose, ovoid/obovoid and funnel shaped).

Anemophilous species have peltate or cyathiform stigmatic surface areas which are large in relation to the rest of the flower. Stigmas protrude beyond the corolla irrespective of flower shape. Large quantities of pollen are shed once stigmas have lost receptivity (protogynous). The floral shape of wind pollinated-species does not differ from insect-pollinated species.

Structural-functional relationships of pollen grains

Angiosperm pollen grains are structurally highly variable (Heslop-Harrison, 1976; Muller, 1979), although certain

architectural features remain relatively consistent throughout. The subdivision of the wall into an exine and intine and the basic structure of the exine being either collumellate or baculate are features distinguishing angiosperm pollen grains (Heslop-Harrison, 1976). These general features are interpreted as having an adaptive significance related to specific functions. Muller (1979) states that the structure of the exine is a compromise between the protective, harmomegathic and reservoir function.

Protection

The development of a foot layer and having sporopollenin as the major structural component (Blackmore and Barnes, 1986) renders an impermeable, resistant barrier to physico-chemical and biological break down (Heslop-Harrison, 1976). The exine therefore performs a highly efficient protective function.

Harmomegathy

"Harmomegathus" is defined as an organ or mechanism which allows a semi-rigid exine to change in volume and harmomegathy is the volume-change process (Wodehouse as cited by Thanikaimoni, 1986). Pollen grains at the time of dispersal only contain 10 - 20% of their possible water content (Heslop-Harrison, 1976). This desiccation process of the vegetative cell is essential for life on land since it allows for the extended survival of pollen grains once

detached from the water containing parent. The exine is adapted to accommodating volume changes in three ways:

1. The inherent ability of the pollen wall to contract and stretch as the need arises. This may or may not involve apertural areas (Blackmore and Barnes, 1986). In instances where apertures have a harmomegathic function a sculptured and fully stratified exine may be absent in the area of the colpus (Heslop-Harrison, 1976).
2. Folding of the pollen wall. This may be localized to apertural regions or to non-apertural areas where walls are less thick (apomorphic state).
3. Compression of internal spaces such as cavae within the pollen wall (Blackmore and Barnes, 1986).

These harmomegathic elements may act in combination which result in the great diversity of harmomegathic mechanisms.

Reservoir function

The cavities in the pollen grain wall are adapted to carry physiologically active proteins of sporophytic (exine) or gametophytic (entine) origin. The enzymes function in intraspecific compatibility control and stigma penetration and early growth of the pollen tube respectively. Tapetum derived materials stored also have a function in producing an adhesive surface ("pollenkitt") (Muller, 1979).

Aptations of the exine for dispersal by specific vectors

Apart from the abovementioned adaptations of the pollen wall vital to reproduction *per se*, there is growing concensus that surface ornamentation may be secondarily influenced by the type of pollinator involved (Ferguson and Skvarla, 1982; Crane, 1986; Punt, 1986).

Generalized associations between exine ornamentation and pollination syndromes have been described. The ideally apted grain for wind pollination should be small, light, smooth and not sticky since the form of the exine of wind transported pollen is influenced by aerodynamic considerations (Crane, 1986).

Pollen of entomophilous species are favoured by rougher exine surfaces and the presence of pollenkitt to facilitate adherence to pollinator body surfaces. Rougher surfaces are provided by many different types of surface processes (Punt, 1986).

Although Punt (1986) states that it is not yet clear what influence birds as pollinators have on the ornamentation of grains, Ferguson and Skvarla (1982) found that a number of ornithophilous species of the subfamily Papilionoideae have coarse regulate/verrucate ornamentation. The exine of these species also has a complex layer of tectal collumellae. The speculated function of the latter is to increase the space accommodating surface materials (Heslop-Harrison, 1976).

This aptation, together with a coarser surface allowing an increased surface area for pollenkitt to adhere to, renders the grain more sticky. Sticky pollen grains is a characteristic often associated with bird-pollinated species (Ferguson and Skvarla, 1982). Pollen grains of ornithophillous species are also often found to be larger.

METHODS AND MATERIALS:

Forty *Erica* species were selected from a list published as an appendix to "Pollination syndromes of *Erica* species in the south western Cape" (Rebelo, Siegfried and Oliver, 1985). Equal proportions in relation to the pollination syndromes were selected and it was ensured that as many sections as possible were included.

All pollen samples were collected from specimens kept in the Bolus herbarium (See Appendix 1). Six to ten buds were collected from one herbarium sheet. Only buds developed to a stage just prior anthesis were used. The date of pollen collection and name of the collector were written on each sheet from which material was removed.

Anthers were dissected out and placed into 10ml glass centrifuge tubes. As little extraneous material other than anthers as possible was placed in the tubes. The anthers were gently crushed to release the pollen grains and larger pieces were removed. After each of the following steps tubes

were centrifuged at 2800 RPM for three minutes and then decanted.

1. Seven ml of KOH (10% solution) was added and allowed to stand in a water bath at 50 degrees Celsius for 10 minutes.
2. Rinsed with distilled H_2O.
3. Rinsed in acetic acid.
4. Seven ml of acetolysis mixture was added (9 parts acetic acid + 1 part H_2SO_4) and allowed to stand in water bath at 50 degrees Celsius for 10 minutes.
5. Rinsed in acetic acid.
6. Washed in distilled water twice.
7. Purified batches were either stored in distilled water overnight or slide preparation commenced immediately.

If stored overnight the distilled H_2O was decanted and centrifuge tubes were turned upside-down onto blotting paper. Once drained a 1 x 1 x 0,5mm piece of glycerol jelly was used to collect pollen grains from the bottom of the tube. The pollen encrusted glycerol cube was placed on a slide and covered with a cover slip. A mild heat was used to melt the glycerol and paraffin wax which functions as a sealant. [Excessive heat causes the glycerine to boil which results in an opaque slide since the glycerol and wax intermingles].

Three replicate slides were made of each species. A number (following the U.C.T. systematics laboratory system of slide enumeration), species name, collector's name and slide preparer's name was written on each slide.

The remaining residue to be used for SEM work was stored in 100% ETOH.

Aluminium microscope stubs were covered in a mixture of graphite and glue and allowed to dry for five minutes. A pipette was used to place a drop of pollen in 100% ETOH onto the stub and allowed to evaporate. Stubs were coated in Au/Pd and stored under vacuum prior to scanning.

The slides which were prepared were viewed under a Zeiss Axioscope photomicroscope. Two views of tetrads were photographed at 1000 times magnification under oil immersion on Ilford FP4 film. It was attempted to photograph hydrated tetrads only.

The size of tetrads were also measured under a light microscope. Two measurements were taken on each tetrad (Fig. 1a and 1b) at 400 times magnification. These measurements were replicated ten times in order to obtain an average size for each species.

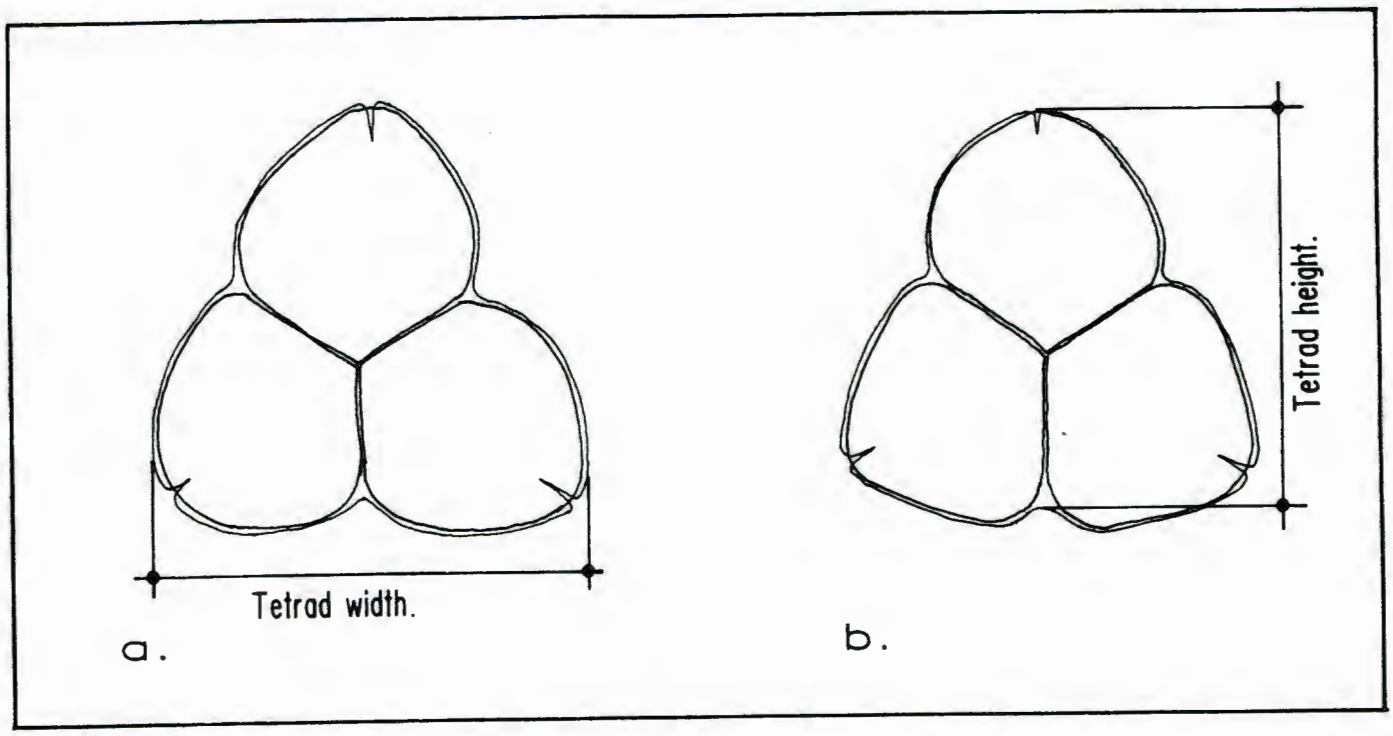


Fig. 1 Showing method of tetrad width and height measurement under LM.

A Student's T-test was used to test any significant size difference between pollen grains from the different pollination syndromes.

The volume of each tetrad was calculated as a measure of size. The formula for the volume of a pyramid with a triangular base is 1/3b times h. The base(b) is the area of a triangle which was calculated as 1/2b times h. Measurement (a) is the base length and (b) the height. Since it was observed that the four grains forming the tetrads are all of similar size, the height measurement in the formula for the volume of a pyramid was regarded as being equal to the height measurement in the formula for a triangle.

A cluster analysis was done using ~~the Bolus Herbarium~~ Uniter group maker and structure analyzed ~~programme~~. An 80% minimization of effect of group size in clustering was selected. Four properties/characters were used in the analysis, being: the exine sculpturing type, the type of aperture margin, tetrad width and tetrad shape. The other characters studied were excluded due to the lack of meaningful variation or to avoid the overemphasis of any particular aspect of any one trait (eg. the inclusion of volume and or tetrad height would overemphasize the size of pollen grains in the analysis). A dendrogram was drawn to show the clustering of species and linkages were indicated as a percentage similarity.

RESULTS:

Pollen unit and tetrad cohesion:

All species in this study except *E. nudiflora* shed their pollen grains in tetrads (Table 2).

A further character looked at was the type of cohesion which keeps compound pollen together. LM reveals that all species appear to have simple *calymmate* type of cohesion, where the grains are fused together by their tectum which surrounds the entire tetrad without interruption (Roland cited by Knox and McConchie, 1986) (Fig. 2).

The fact that only *E. nudiflora* has pollen grains shed in monads is not indicative of any trend within the confines of this study. *E. rubiginosa* which is classified into the same section sheds pollen in tetrads. This would seem to indicate that this character is not one which is consistent within sections. Erdtman (1943) shows that pollen is shed in monads by other Minor Genera (*Bruchenthalia*), as well as in *Erica* spp. This would discredit the suspicion that the monads observed in this study are possibly an artifact of preparation techniques.

Calymmate tetrads are not confined to the genus *Erica* only since Knox and McConchie (1986) found *Rhododendron* also displayed this form of cohesion. A TEM study would however

Table 2 List of *Erica* species studied. Subgenus and section to which each species belongs is indicated. Vector, pollen unit, tetrad shape and aperture margin of each species is also shown.

SPECIES NAME	SUBSPECIES	SECTION	VECTOR	POLLEN UNIT	APERTURE MARGIN	TETRAD AMB
E. plukeneti		Gigandra	Bird	Tetrad	Margo	Clover
E. coccinea			Bird	Tetrad	Margo	Clover
E. viridiflora		Didymanthera	Bird	Tetrad	Distinct	Clover
E. sessiliflora		Pleurocallis	Bird	Tetrad	Distinct	Clover
E. pinea			Bird	Tetrad	Distinct	Subclover
E. nevillei			Bird	Tetrad	Margo	Clover
E. curviflora	Syringodea	Evanthe	Bird	Tetrad	Distinct	Clover
E. discolor			Bird	Tetrad	Margo	Clover
E. vestita		Pleurocallis	Bird	Tetrad	Distinct	Subtriangular
E. perspicua		Evanthe	Bird	Tetrad	Margo	Subtriangular
E. pillansii			Bird	Tetrad	Distinct	Subclover
E. cerinthoides		Dasyanthes	Bird	Tetrad	Distinct	Clover
E. massonii		Bactridium	Bird	Tetrad	Margo	Clover
E. retorta		Euryloma	Insect	Tetrad	Margo	Supraclover
E. infundibuliformis	Stellanthe	Callista	Insect	Tetrad	Margo	Clover
E. glutinosa		Myra	Insect	Tetrad	Distinct	Supraclover
E. hirtiflora		Ephebus	Insect	Tetrad	Margo	Subglobular
E. filiformis		Ceramia	Insect	Tetrad	Margo	Clover
E. ocellata			Wind	Tetrad	Distinct	Subglobular
E. rubiginosa		Gypsocallis	Wind	Tetrad	Margo	Supraclover
E. nudiflora			Insect	Monad	Distinct	
E. blandfordia		Orophanes	Insect	Tetrad	Distinct	Subglobular
E. chionophylla			Wind	Tetrad	Distinct	Subglobular
E. campanularis		Leptodendron	Insect	Tetrad	Distinct	Subclover
E. pulchella		Hermes	Insect	Tetrad	Distinct	Subclover
E. coarctata	Euerica	Chlorocodon	Wind	Tetrad	Distinct	Subclover
E. hispidula		Arsace	Wind	Tetrad	Distinct	Subglobular
E. leucopelta			Wind	Tetrad	Margo	Clover
E. leptopus			Insect	Tetrad	Distinct	Subclover
E. tenuis			Insect	Tetrad	Distinct	Subclover
E. copiosa			Wind	Tetrad	Distinct	Clover
E. microcodon			Wind	Tetrad	Margo	Supraclover
E. setacea			Wind	Tetrad	Distinct	Clover
E. imbricata		Geissostegia	Insect	Tetrad	Distinct	Subtriangular
E. lasciva		Elytrostegia	Insect	Tetrad		Supraclover
E. holosericea	Chlamydanthe	Eurystegia	Insect	Tetrad	Distinct	Globular
E. baccans		Trigemma	Insect	Tetrad	Distinct	Globular
E. peltata		Polycodon	Wind	Tetrad	Distinct	Subclover
E. calycina	Platystoma	Eurystoma	Insect	Tetrad	Distinct	Clover
E. cristaeflora		Melastemon	Insect	Tetrad	Distinct	Clover

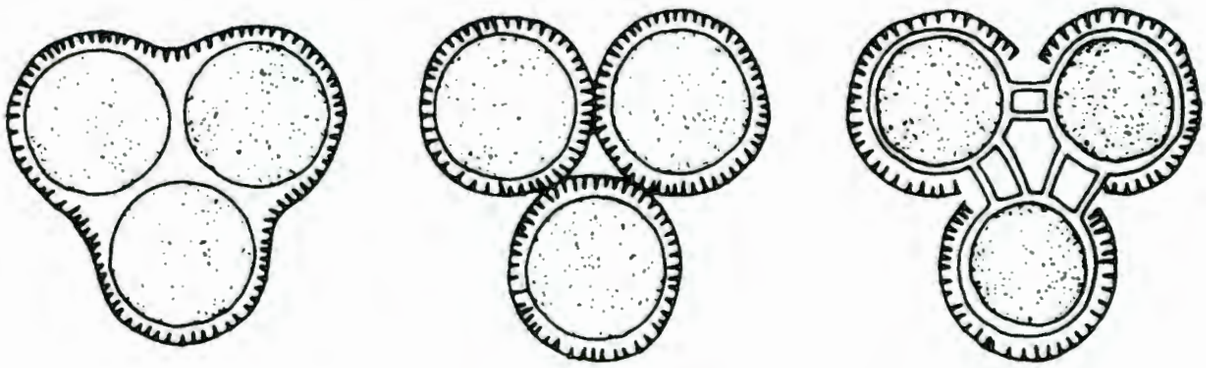
elucidate the correctness of initial interpretation based on LM (light microscopy). Such a study may well prove this character to be of phylogenetic importance below family level in the *Ericaceae*. Niezgoda (cited by Knox and McConchie 1986) found that calymmate and acalymmate (connected by wall bridges) type polyads occur within the genus *Calliandra* (Mimosoideae). The possibility therefore exists that species in certain unexamined sections display acalymmate tetrads.

Tetrad shape:

The outline of a pollen grain as seen in polar view with the axis directed towards the observer is termed the pollen amb. Specific terminology exists which describes the major pollen amb types (Walker and Doyle, 1975). Distinct differences in the outline of tetrads are evident when viewed along the polar axis of any one of the grains. A terminology exists to describe the outline or "amb" of tetrads (Foss and Doyle, 1988) (Fig. 3). Distinction between shapes in the study of Foss and Doyle are relatively coarse. Three intermediate shapes between globular and subglobular (Fig. 3) were observed under the LM and from light micrographs. A more refined description and terminology of the six different shapes recognized in this study is therefore proposed. It is also suggested that the term subglobular does not aptly describe the particular shape in question and that the term

CALYMMATE

ACALYMMATE



CONTINUOUS TECTUM AT JUNCTIONS

TECTUM DISCONTINUOUS AT JUNCTIONS

Fig.2 Stylized diagram showing type of cohesion which keeps compound pollen together (ex Knox and McConchie, 1986).

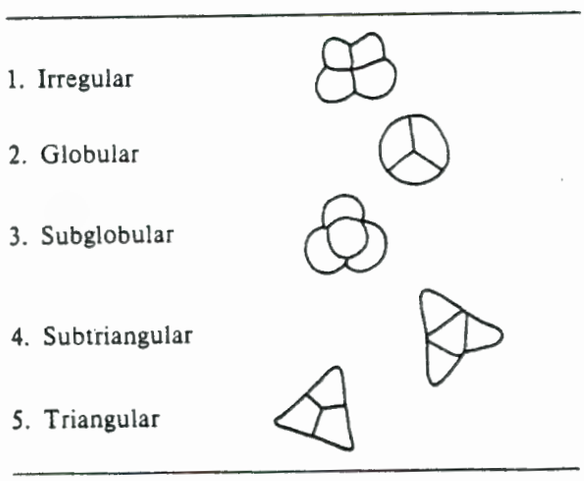


Fig. 3 The major tetrad shapes distinguished in the study of Irish *Ericas* by Foss and Doyle (1988).

"clover shaped" be given. The term subglobular is however retained, but describes a different shape.

The six shapes recognized are therefore: supraclover, clover, subclover, subtriangular, globose and subglobose (Fig. 4). The distinction relates to the ~~amount~~ degree of tectum indentation where two grains meet and whether individual grains are rounded or form an apex in the vicinity of the aperture. Supraclover tetrads have very distinct individual grains. Indentations where grains meet are acutely pronounced. The edges of grains are well rounded in these areas but form semi-acute apices in the apertural regions (Fig. 4c and Fig. 11a). The grains of clover tetrads are also distinct but indentation between grains are not as pronounced as in the former tetrad shape. Where grains meet each grain is less rounded and apertural regions are no longer semi-acute but more rounded (Fig. 4a and Fig. 9a). Subclover tetrads show less distinct individual grains yet are still identifiable. There is only a slight indentation where grains meet and apertural regions are well rounded (Fig. 4b and Fig. 10c). The term subglobular is applied to tetrads in which the outline of individual grains are only just recognisable. There is slight indentation where grains meet and the outline of the entire tetrad is almost round (Fig. 4e). The shapes globular and subtriangular ~~collate~~ agree closely ~~well~~ with those of Foss and Doyle (1988) and are

consequently used in this study (Fig. 4d and Fig 4f).

Bird-pollinated species display relative uniformity, 77% being clover shaped. Only *E. vestita* is markedly different and is subtriangular. The two other syndromes show a greater diversity in shapes. Entomophilous species range from supraclover (*E. retorta*) (Fig. 4c) to globular (*E. baccans*) (Fig. 4f) and from supraclover (*E. rubiginosa*) (Fig. 12c) to subglobular (*E. hispidula*) (Fig. 4e) in anemophilous species.

With the exception of section *Arsace* where shapes range from subglobular (*E. hispidula*) (Fig. 4e) to supraclover (*E. microcodon*) (Table 2) variation within sections where two or more species were studied is minimal. Species within each of the respective sections *Gigandra, Ceramia* and *Orophanes* show similar shapes whereas those in *Pleurocallis* and *Evanthe* vary little, being either clover or subclover.

Tetrad size:

Statistically bird pollinated species have tetrads of a larger volume than the other syndromes. Insect and wind pollinated species are not significantly different (Table 4).

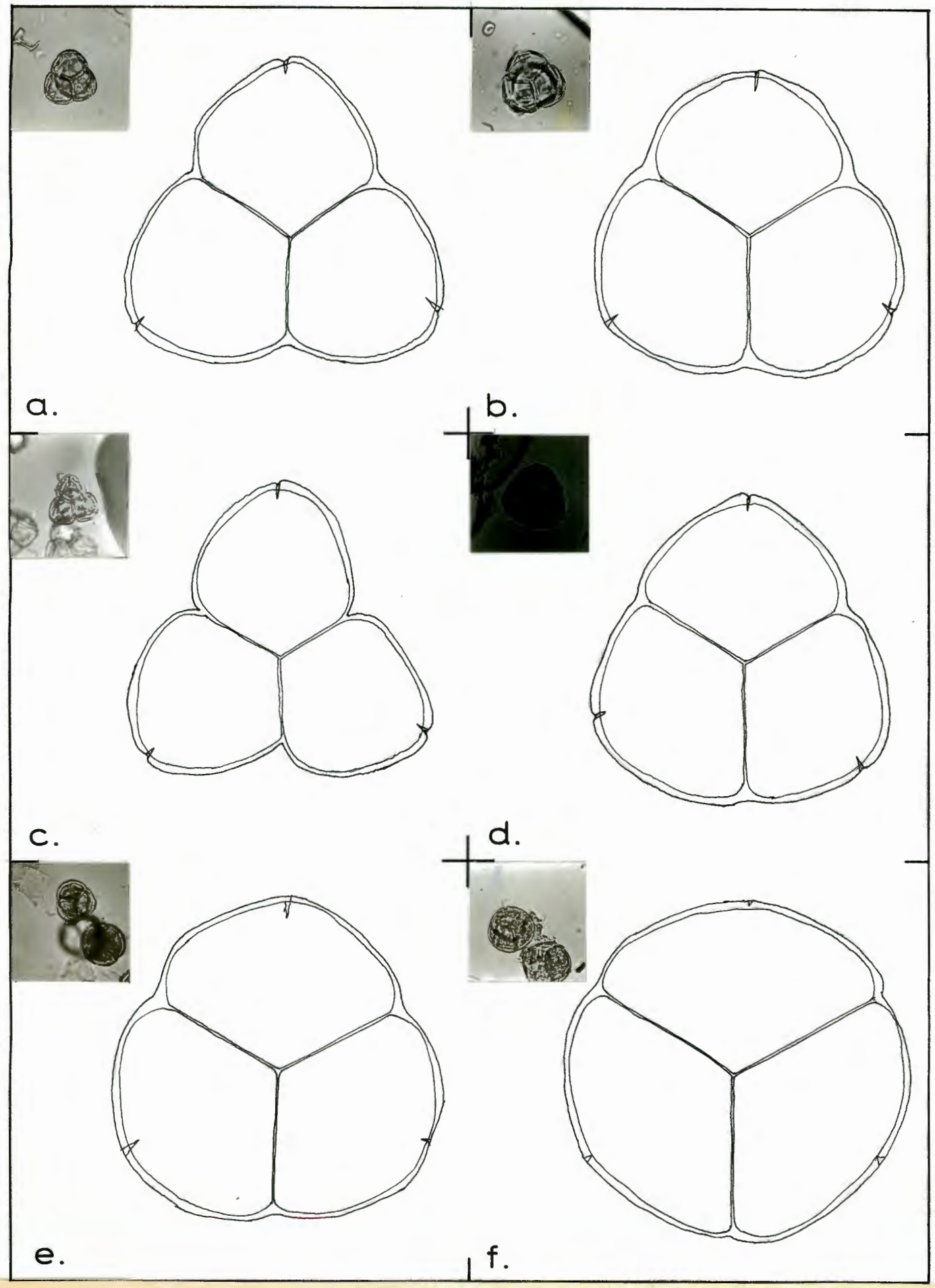


Fig.4 Light micrographs and outline drawings of tetrad shapes as distinguished in this study. (a) Clover shape of *E. curviflora* (b) Subclover tetrad of *E. pillansii* (c) Supraclover shape of *E. retorta* (d) Subtriangular as found in *E. vestita* (e) Subglobular of *E. hispidula* (f) Globular as found in *E. baccans*.

Table 3 The tetrad height and width measured in um, the basal area in um^2 and volume in um^3, as well as the type of exine sculpturing of each *Erica* species studied.

SPECIES NAME	SECTION	TETRAD HEIGHT	TETRAD WIDTH	BASAL AREA	VOLUME	EXINE SCULPTURING
E. plukeneti	Gigandra	36.23	37.1	672.07	8035.16	Fossulate
E. coccinea		37.23	37.23	693.04	8514.58	Fossulate
E. viridiflora	Didymanthera	40.93	41.57	850.73	11490.73	Psilate
E. sessiliflora	Pleurocallis	38.76	40.55	785.86	10051.77	Psilate
E. pinea		58.39	60.18	1756.96	33854.24	Irregularly micro-verrucate
E. nevelei		47.81	49.21	1176.37	18559.86	Fossulate
E. curviflora	Evanthe	38.26	41.31	790.26	9977.67	Irregularly verrucate
E. discolor		37.11	38.6	716.22	8771.08	Micro-verrucate
E. vestita	Pleurocallis	46.4	48.96	1135.87	17392.47	Psilate
E. perspicua	Evanthe	42.46	44.25	939.43	13163.07	Irregularly micro-gemmate
E. pillansii		43.86	47.94	1051.32	15216.66	Psilate
E. cerinthoides	Dasyanthes	44.63	46.79	1044.12	15377.68	Fossulate
E. massonii	Bactridium	43.99	47.68	1048.72	15223.98	Irregularly verrucate
E. retorta	Euryloma	35.58	40.54	721.21	8467.98	Irregularly verrucate
E. infundibuliformis	Callista	40.67	43.1	876.44	11762.77	Micro-verrucate/fossulate
E. glutinosa	Myra	41.44	42.97	890.34	12175.56	Micro-verrucate/gemmate
E. hirtiflora	Ephebus	32.18	33.71	542.39	5759.90	Irregularly verrucate
E. filiformis	Ceramia	34.86	36.98	644.56	7414.91	Irregularly verrucate
E. ocellata		39.52	40.93	808.78	10547.74	Irregularly scabrate/verrucate
E. rubiginosa	Gypsocallis	33.18	34.7	575.67	6303.27	Irregularly verrucate
E. nudiflora		19.25	17.09	164.49	1044.93	Psilate
E. blandfordia	Orophanes	39.78	40.8	811.51	10653.04	Micro-fossulate
E. chionophylla		30.61	30.74	470.48	4752.42	Micro-scabrate
E. campanularis	Leptodendron	43.99	44.37	975.92	14167.11	Scabrate
E. pulchella	Hermes	43.61	45.26	986.89	14202.69	Micro-scabrate/verrucate
E. coarctata	Chlorocodon	26.52	27.8	368.63	3226.08	Micro-scabrate/verrucate
E. hispidula	Arsace	34.09	34.6	589.76	6634.59	Micro-verrucate/scabrate
E. leucopelta		23.72	25.38	301.01	2356.16	Micro-verrucate
E. leptopus		25.76	26.39	339.90	2889.45	Micro-verrucate/scabrate
E. tenuis		23.33	24.22	282.53	2175.14	Micro-verrucate/scabrate
E. copiosa		28.31	30.48	431.44	4030.68	Micro-verrucate/gemmate
E. microcodon		31.8	33.46	532.01	5582.95	Micro-verrucate/scabrate
E. setacea		26.39	27.54	363.39	3164.66	Micro-scabrate/verrucate
E. imbricata	Geissostegia	31.41	33.21	521.56	5406.16	Psilate
E. lasciva	Elytrostegia	29.07	30.09	437.36	4195.62	Irregularly scabrate
E. holosericea	Eurystegia	45.5	46.41	1055.83	15853.25	Irregularly scabrate
E. baccans	Trigemma	36.41	37.86	689.24	8281.44	Irregularly scabrate
E. peltata	Polycodon	27.16	28.05	380.92	3414.10	Micro-verrucate/scabrate
E. calycina	Eurystoma	35.49	36.85	653.90	7658.32	Gemmate
E. cristaeflora	Melastemon	22.95	23.21	266.33	2017.09	Psilate

Table 4 A T-test showing the variation in mean tetrad size of species from three different pollination syndromes

Pollination syndrome	N	x	SD	t	P
Ornithophyly	13	14279.15	6575.78		
Entomophily	16	7350.37	4987.80	3.2	> 0.01
Entomophily	16	7350.37	4987.80		
Anemophily	10	5001.26	2291.48	1.39	< 0.01

Although the range in size of bird pollinated species is large (Table 5), the result is markedly influenced by the very much larger tetrads of *E. pinea* (Table 3).

Table 5 The range in tetrad size of species in different pollination syndromes

Pollination syndrome	Range
Ornithophilous	33854--8771
Entomophilous	15853--2017
Anemophilous	10548--2356

There is much overlap in tetrad size between syndromes. The largest wind pollinated species *E. ocellata* is larger than the smallest bird pollinated species *E. plukeneti*. Similarly a number of entomophilous species have larger tetrads than the smaller ornithophilous species (*E. campanularis* vs *E. viridiflora* (Table 3)).

Apertures:

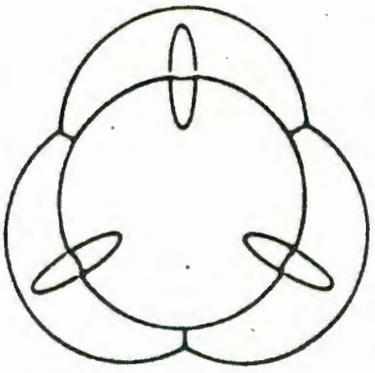
Each grain forming the tetrad in all species have three apertures which are colpate in shape (i.e. tricolpate). All apertures are placed interradially (Fig. 5).

The aperture margins of certain species show a change in exine sculpturing in the vicinity of the aperture which is termed as having a *margo* (Thainikaimoni, 1986) (*E. nevillei*) whilst others are sculptured to the edge of the aperture and show a distinct limit between the apertural and the non-apertural regions (*E. ocellata*) (Fig. 6a and 6b). There is however no pattern which may relate the presence or absence of a margo to either the pollination syndrome or section into which species are classified (Table 2).

Those aperture characters which show no alternative states throughout the species studied namely: all being tricolporate and equatorially and interradially placed, are characters which can only be used at higher taxonomic levels. Consequently these are of no use in the present study.

Exine sculpturing:

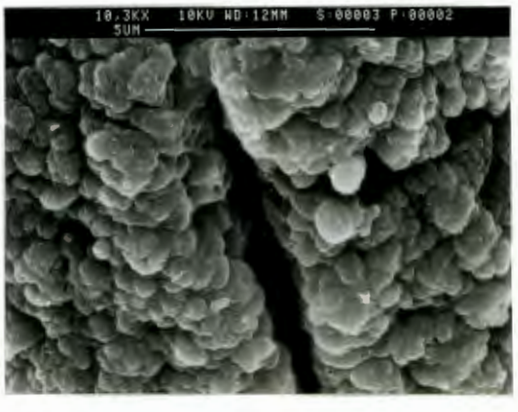
A number of sculptural types occur within the genus (Table 3), varying from psilate (smooth) to verrucate (wart like) (named according to Traverse, 1988) (Fig. 7 and Fig. 8). Many surfaces are not heavily ornamented and sculptural elements, though resolvable as a particular sculptural type, are smaller than 1um and are given the prefix *micro* (as per Traverse 1988). Those species which have psilate and microsculptured surfaces together form 55% of the species studied. The remaining 45% have sculptural elements only



interradially placed aperture

Fig. 5 Diagram showing interradially placed apertures of tetrahedral tetrads found in all *Erica* species in this study.

a.



b.

Fig.6 Apertural regions of *E. nevillei* and *E. ocellata* grains showing distinction between those in which an exine structure change occurs (margo) and those in which sculpturing continues to the edge of the aperture (distinct) respectively.

A. Positive sculptural elements absent
  B. Surface smooth — *psilate*
  BB. Diameter of pits <1 µm — *micropitted*
  BBB. Surface pitted, diameter of pits ≥1 µm — *foveolate*
  BBBB. Surface with grooves — *fossulate*
  (includes *negatively reticulate*)

AA. With positive sculptural elements
  B. Sculptural elements approximately isodiametric along the surface of the palynomorph (but may extend upward)
    C. No dimensions ≥1 µm — *scabrate*
    CC. At least one dimension ≥1 µm
      D. Sculptural elements not pointed
        E. Lower part of element constricted
          F. Greatest diameter along surface of palynomorph equal to or greater than height of element: elements globular — *gemmate*

          FF. Height of element greater than greatest diameter of projection: elements club-shaped — *clavate*

        EE. Lower part of element not constricted
          F. Greatest diameter along the surface of palynomorph equal to or greater than height of element: elements wart-like — *verrucate*

          FF. Height of element greater than greatest diameter of projection: elements rod-shaped — *baculate*

      DD. Sculpturing elements pointed — *echinate*

  BB. Sculptural elements elongated along the surface of the palynomorph (length at least twice the breadth)
    C. Elements irregularly distributed — *rugulate*
    CC. Elements approximately parallel to each other — *striate*
  BBB. Sculpturing elements forming a reticular (net-like) pattern — *reticulate*
  (elements <1 µm but resolvable as reticulum: *microreticulate*)

Fig. 7 A description and illustration of the major sculptural types of pollen and spores as described by Traverse (1988).

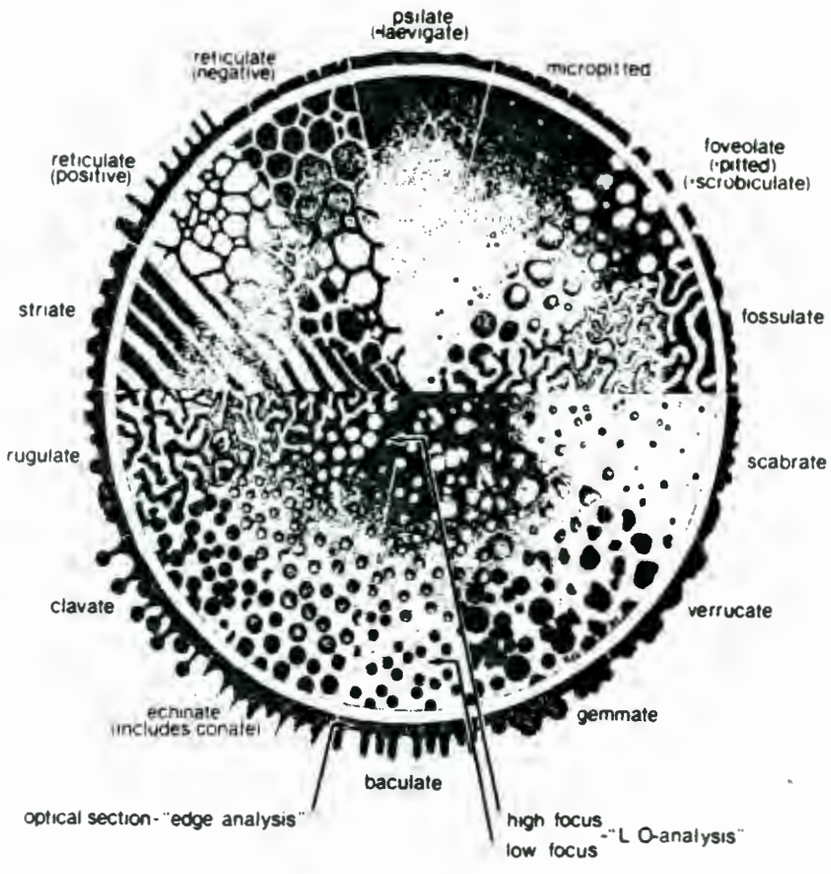


Fig. 8 Sculpture types as seen at various levels of focus with LM. High focus is shown towards the inside of the illustration and low focus towards the outside. By comparing these levels the sculpture can be analysed. This is termed "LO analysis". Focussing on the surface of the exine at the outer edge of the grain at mid-focus is called edge analysis and provides a check on conclusions made from LO analysis (ex Traverse, 1988).

marginally larger than 1um and very few exceed 2um (Fig. 9e). Many sculptural patterns do not fall into the types as delimited by Traverse but are of intermediate nature (Fig. 9d).

A description of the sculptural types are as follows:

Psilate - entirely smooth surface which may be slightly undulating as an *E. pillansii* (Fig. 10d).

Fossulate - no positive sculptural elements but the surface is grooved (*E. coccinea* in Fig. 9b).

Micro-fossulate - as in fossulate exines with the only difference being that the grooves are smaller, thus forming a pattern more compact in appearance (*E. blandfordia* in Fig. 11d).

Scabrate - positive sculptural elements of which no dimensions are larger than 1um. Elements extend slightly upward as in *E. campanularis* (Fig. 11f).

Irregularly scabrate - differs from the above in that the sculptural elements are of varying sizes (*E. baccans* in Fig. 12b).

Micro-scabrate - as in scabrate exines but sculptural elements are much reduced as seen in *E. chionophylla* (Fig. 13f).

Irregularly verrucate - large wart-like surface projections of irregular size. The lower part of the elements are not constricted and the greatest diameter of any element is

greater than the height of the element (*E. curviflora* in Fig. 9f).

Micro-verrucate - the sculpturing is the same as above, but sculptural elements are reduced below 1um and consequently more compact (*E. pinea* in Fig. 9d).

Scabrate/verrucate - the sculptural elements are neither wart-like nor scabrate but tend to form clusters of sculptural elements which are a combination of the aforementioned sculptural types (Fig. 13b).

Gemmate - positive sculptural elements greater than 1um in any dimension. The lower part of the element is constricted and the greatest diameter is greater than the height of the element. The shape of the elements are globular (*E. calycina* in Fig. 12d).

Micro-gemmate - the intrapertural areas have a gemmate sculpturing but elements are much reduced and more compact (*E. perspicua* in Fig. 10b).

Verrucate/gemmate - elements are neither wart-like nor globular and elements are of varying sizes (*E. copiosa* in Fig. 14d).

Considerable variation of sculptural types occur within any one pollination syndrome. For example, seven exine sculptural types are observed in the thirteen bird-pollinated species examined (Table 3, Fig. 9 and Fig. 10) and those species in the other two syndromes display similar diversity (insect pollinated species in Fig. 11 and Fig. 12

and wind pollinated species in Fig. 13 and Fig. 14). A particular type of ornamentation is also not limited to any one pollination syndrome. Psilate exines for instance are found in bird- and insect-pollinated species (Fig. 9e and 9f and Fig. 12e and 12f).

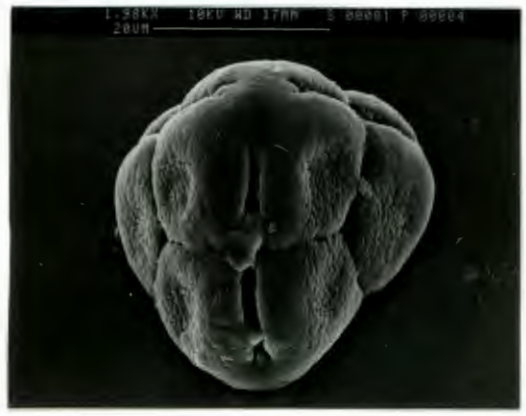
Species within sections also display sculptural variation. Apart from the section Gigandra where *E. plukeneti* and *E. coccinea* both have fossulate sculpturing, all other sections with two or more species studied show large sculptural variability. (See sections *Pleurocallis, Evanthe, Ceramia, Gypsocallis, Orophanes and Arsace* in Table 2).

Exine stratification:

Without having used the proper staining techniques (Faegri as cited by Walker and Doyle, 1975), it is difficult to distinguish between the different layers by means of LM only. By observation of the light micrographs taken at 1000 times magnification, it would appear if there is variation in the thickness of the exine between species. Bird pollinated species appear to generally have thicker exines (Fig. 15a - d) exines as seen in *E. peltata* and *E. infundibuliformis* respectively (Fig. 15l and Fig. 15e).

Cluster analysis:

The fact that only four characters were able to be used is not ideal for such an analysis. However the trend which is revealed by the dendrogram (see Appendix 2) is much as one



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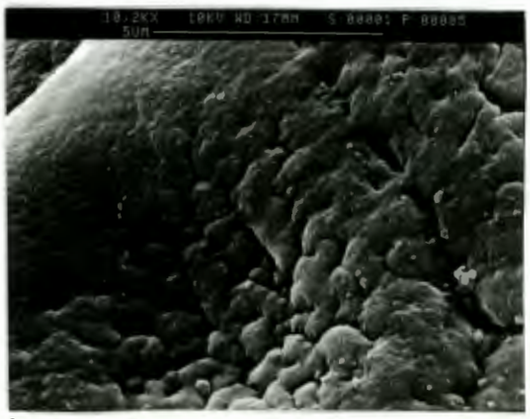

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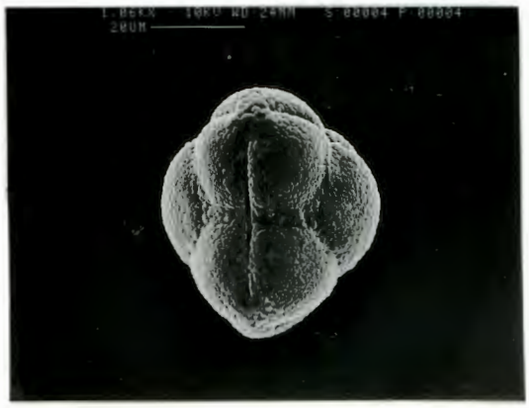

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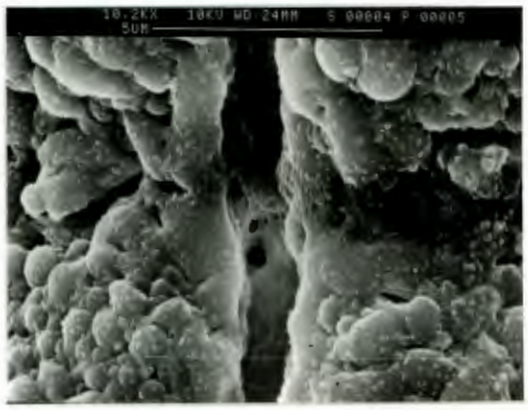

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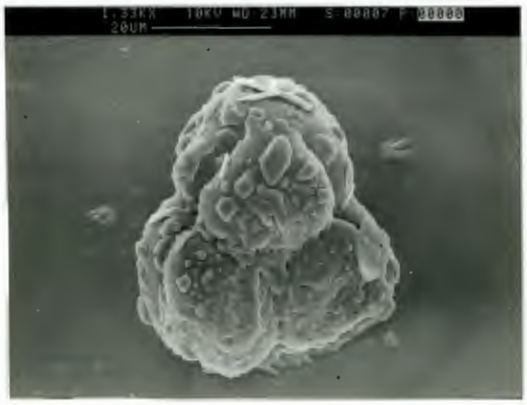

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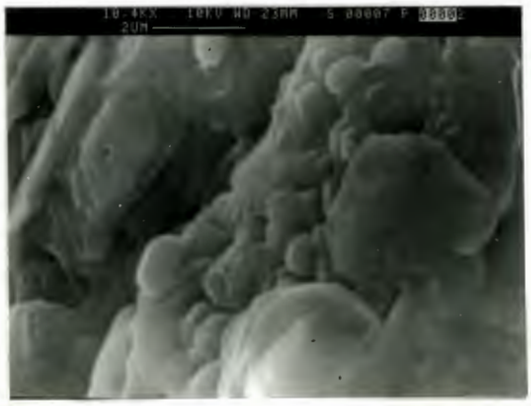
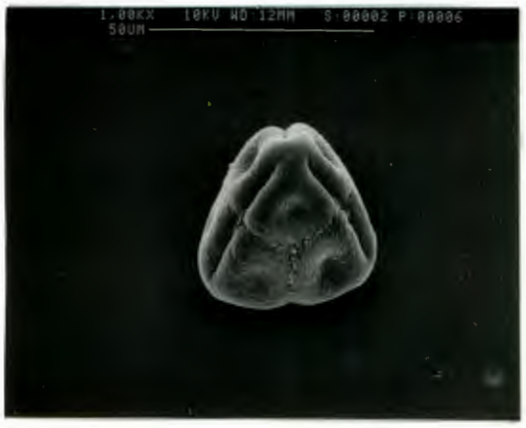


f.

Fig. 9 Tetrad shape and exine pattern of bird-pollinated species (a) Clover shape of *E. coccinea* SEM x 2000 (b) Part of colpal and intercolpal area showing fossulate pattern of intercolpal area of *E. coccinea* SEM x 10000 (c) Subclover shape of *E. pinea* SEM x 1000 (d) Colpal area of *E. pinea* showing no change of micro-regulate/verrucate sculpturing in this area SEM x 1000 (e) *E. curviflora* - clover shape SEM x 1300 (f) Irregularly verrucate pattern of *E. curviflora* in apertural and non-apertural areas SEM x 10000.



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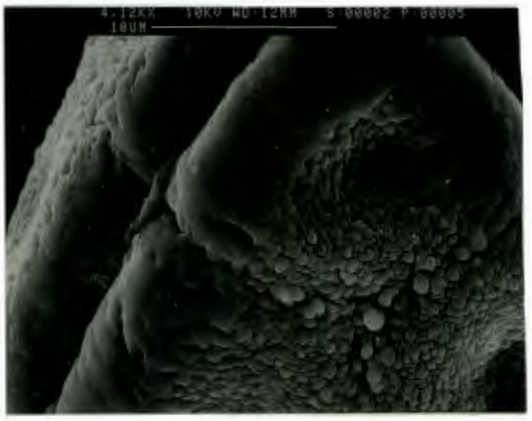

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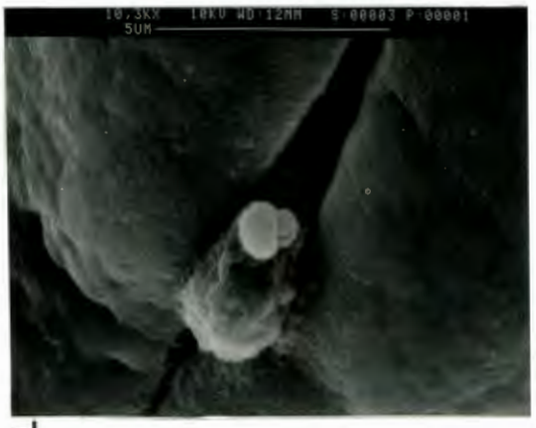

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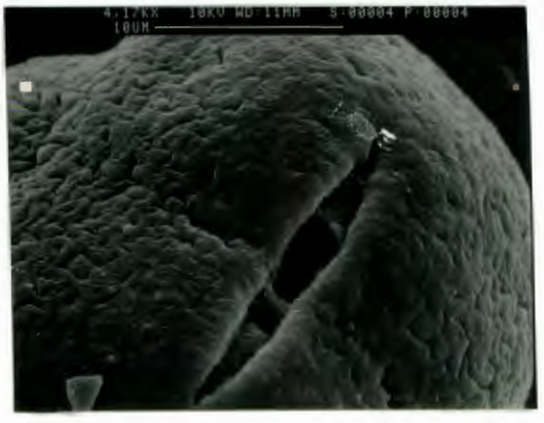
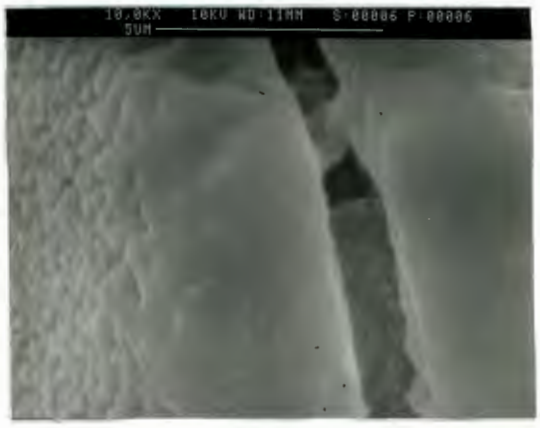


f.

Fig. 10 Tetrads and exine sculpturing of bird-pollinated species (a) Subtriangular tetrad of *E. perspicua* SEM x 1000 (b) Irregularly micro-verrucate pattern of interapertural regions of *E. perspicua* SEM x 4000 (c) Clover tetrad of *E. pillansii* SEM x 1000 (d) Psilate surface of *E. pillansii* extending to apertural regions SEM x 10000 (e) *E. cerinthoides* - subclover shape SEM x 2000 (f) Fossulate pattern of *E. cerinthoides* SEM x 4000.

a.



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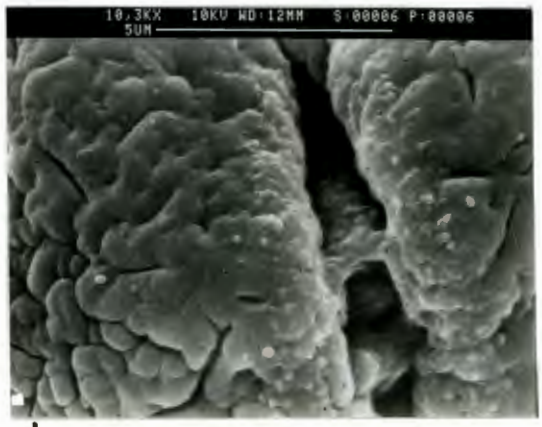

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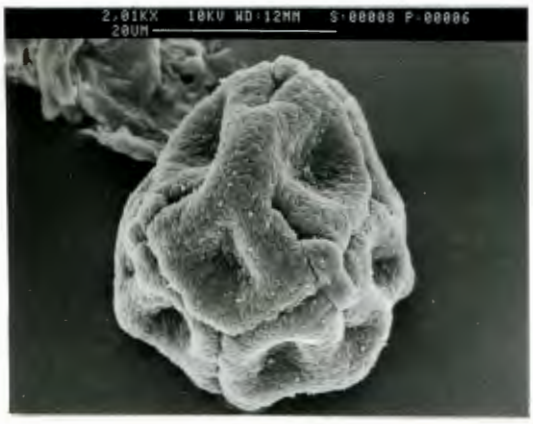

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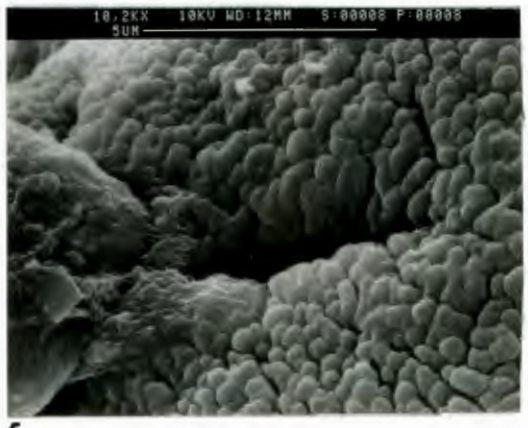
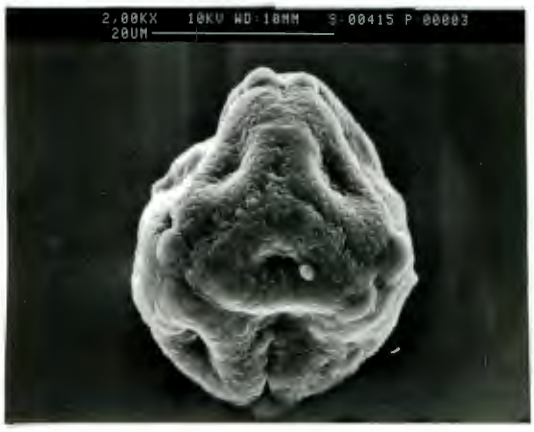


f.

Fig. 11 - Exine pattern and shapes of insect - pollinated species (a) Supraclover shape of *E. retorta* showing individual grains very distinctly SEM x 1000 (b) Colpal and intercolpal areas of an *E. retorta* grain showing a margo and an irregularly verrucate pattern SEM x 10000 (c) *E. blandfordia* - subglobular shape SEM x 2000 (d) Microfossulate exine in apertural region of *E. blandfordia* SEM x 10000 (e) Dehydrated subclover tetrad of *E. canpanularis* SEM x 200 (f) Regulated scabrate pattern of *E. canpanularis* up to apertural region SEM x 10000.



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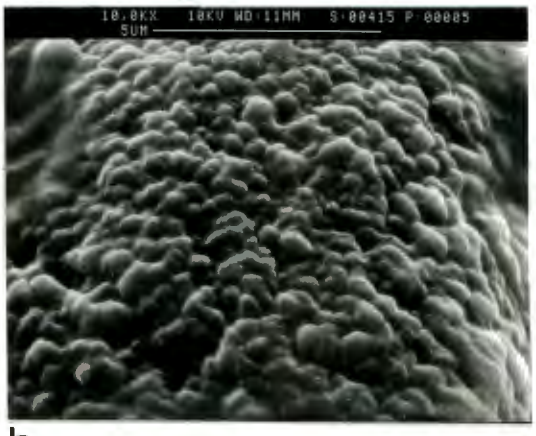

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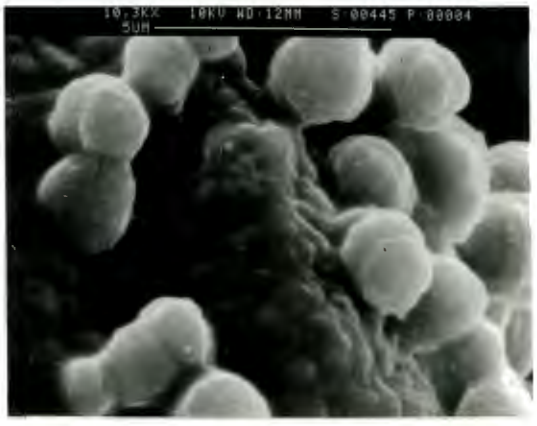

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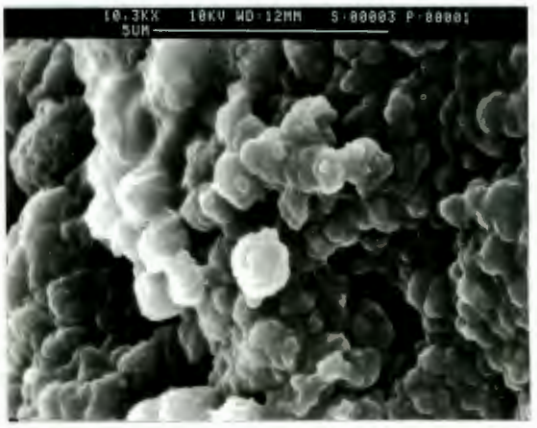
Fig. 12 Insect-pollinated species showing exine pattern and shapes (a) Dehydrated tetrad of *E. baccans* which is globular when hydrated SEM x 2000 (b) Irregularly scabrate surface of *E. baccans* SEM x 10000 (c) Dehydrated tetrad of *E. calycina* which is clover shaped when hydrated SEM x 2000 (d) Distinctly gemmate pattern of *E. calycina* SEM x 10000 (e) *E. cristaeflora* - clover shaped SEM x 3000 (f) Psilate surface of *E. cristaefolia* extending to apertural area SEM x 21000.

a.



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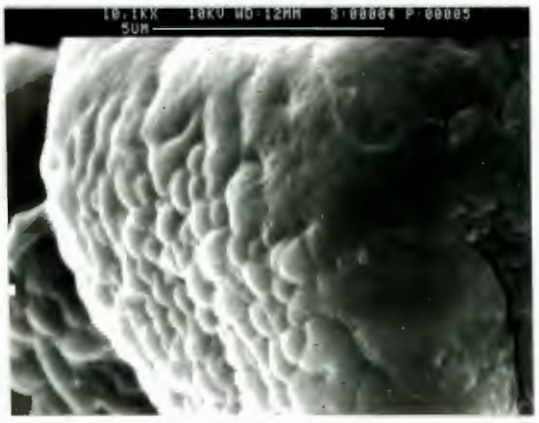

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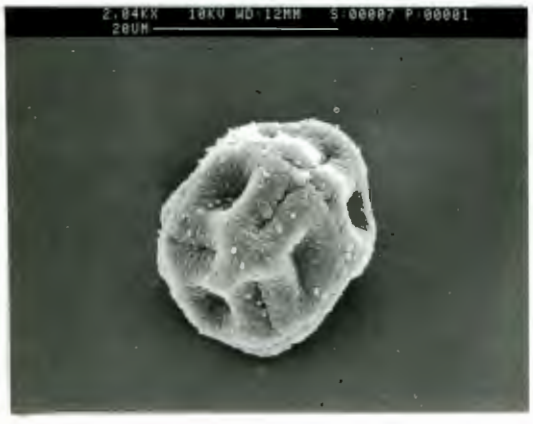

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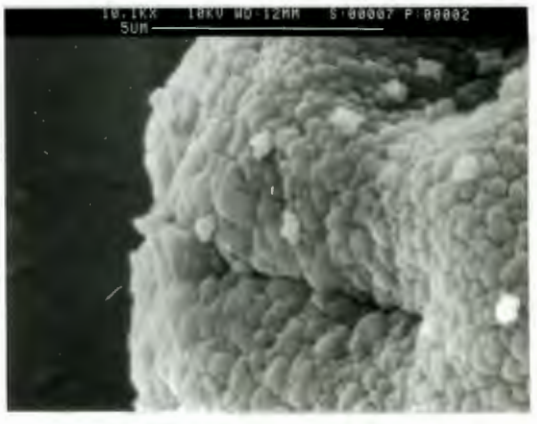
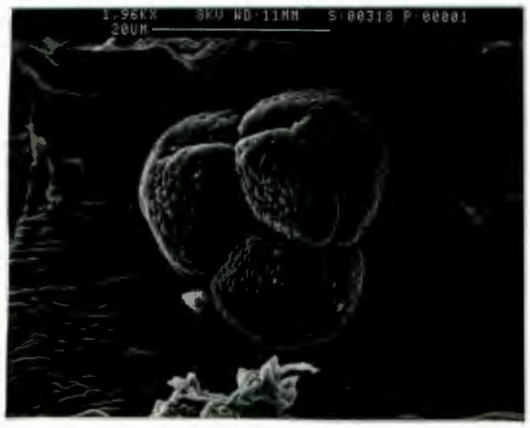


f.

Fig. 13 Tetrad shapes and exine sculpturing of wind-pollinated *Erica* (a) Subglobular shape of *E. ocellata* SEM x 2000 (b) The highly sculptured exine of *E. ocellata* which is irregularly scabrate/verrucate SEM x 1000 (c) *E. rubiginosa* - supraglobular shape with a distinct margo in the apertural region SEM x 3000 (d) Irregularly verrucate intercolpal exine of *E. rubiginosa* (e) Subglobular shape of *E. chionophylla* SEM x 2000 (f) Higher magnification of colpal/intercolpal region of *E. chionophylla* showing microscabrate exine SEM x 10000.



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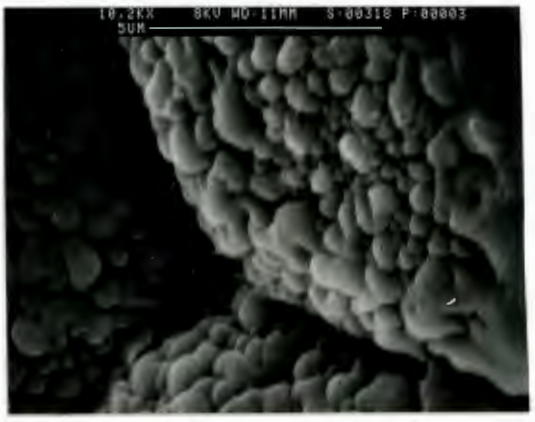

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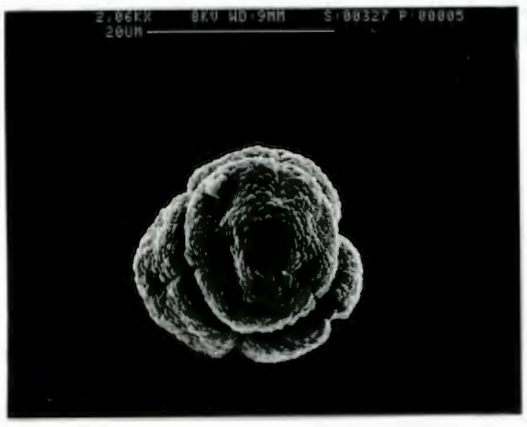
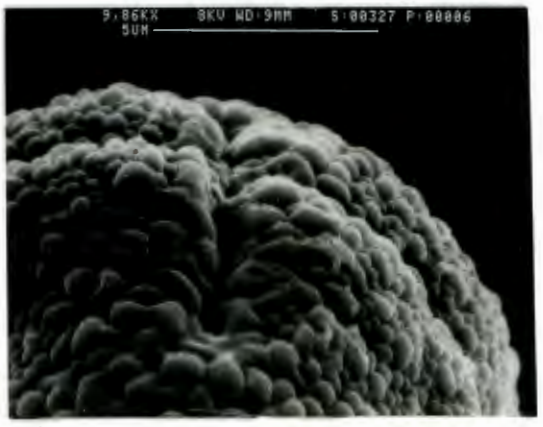
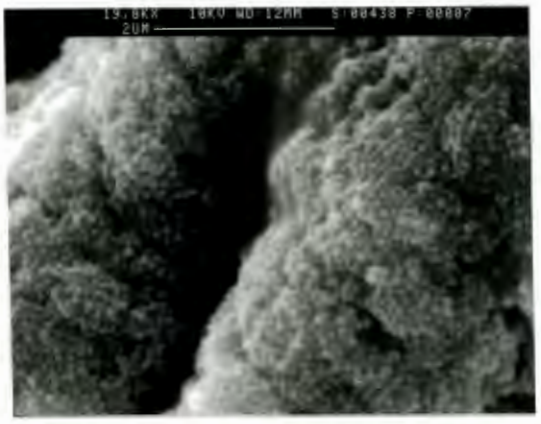


c.



d.

e.



f.

Fig. 14 Exine patterns and shapes of wind-pollinated species (a) Clover shape of *E. leucopelta* SEM x 2000 (b) Micro-verrucate sculpturing of *E. leucopelta* exine SEM x 10000 (c) *E. copiosa* - clover shape SEM x 2000 (d) Colpal/intercolpal areas of *E. copiosa* showing a micro verrucate/gemnate pattern SEM x 10000 (e) Dehydrated tetrad of *E. peltata*, normally subclover shaped SEM x 3500 (f) High magnification showing granular texture of a micro verrucate/scabrate exine pattern of *E. peltata* SEM x 20000.

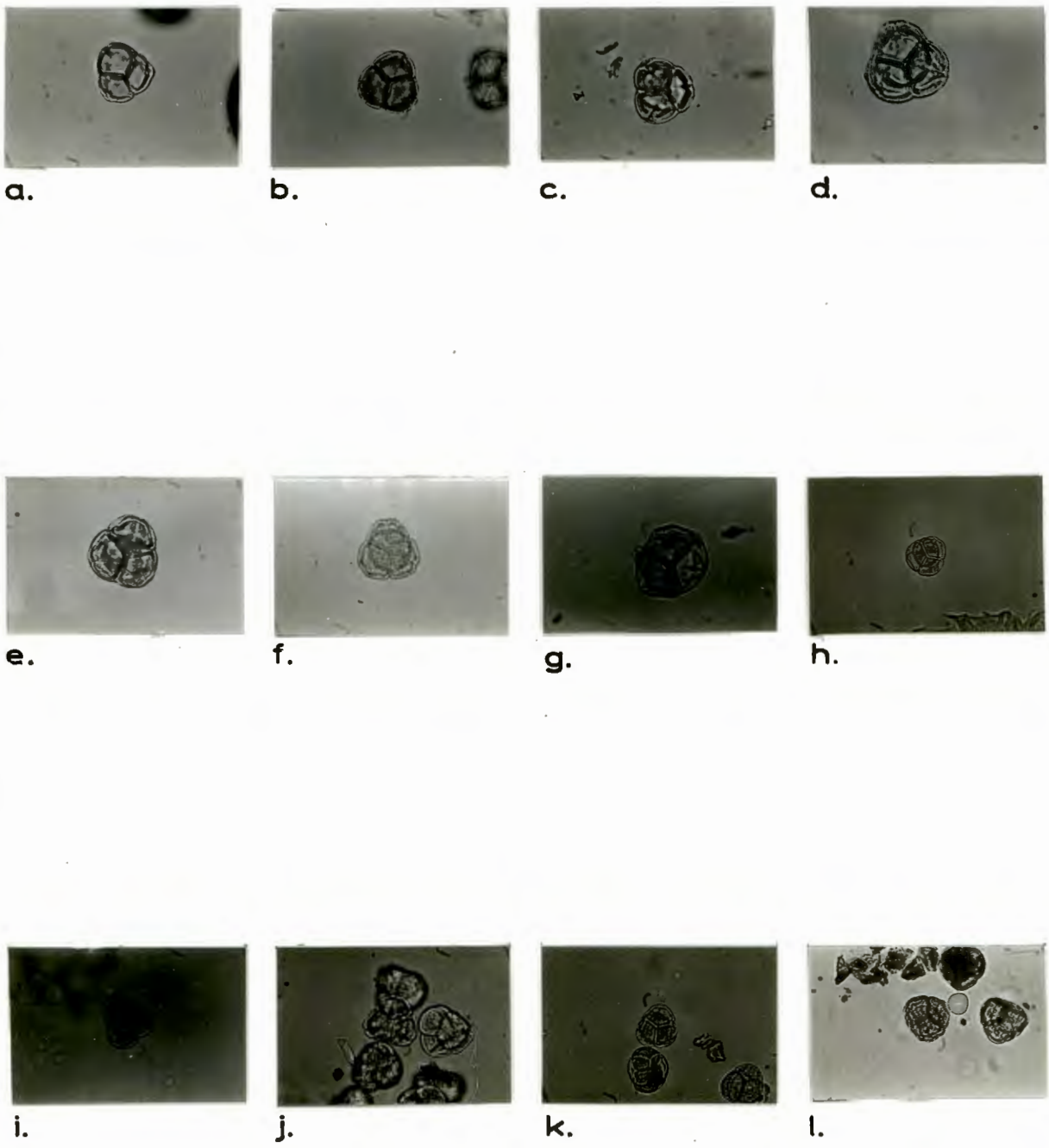


Fig. 15 Light micrographs of bird- (a-d), insect- (e-l) and wind-pollinated Erica species to show exine thickness (a) *E. plukeneti* (b) *E. coccinea* (c) *E. sessiliflora* (d) *E. nevillei* (e) *E. infundibuliformis* (f) *E. filiformis* (g) *E. campanularis* (h) *E. tenuous* (i) *E. rubiginosa* (j) *E. microcodon* (k) *E. setaceae* (l) *E. peltata*. All x 1000.

would expect from superficial viewing of the data. Species which fall within the same section are often linked at a relatively low similarity index. For instance, the species within the section *Pleurocallis* namely *E. curviflora* and *E. pillansi* are linked low down at a 76% similarity index. The species *E. discolor* and *E. perspicua*, which are clustered at a high similarity index (98%) are linked to the other two at the lowest similarity index on the dendrogram (52,5%). *E. plukeneti* and *E. coccinea* (section *Gigandra*) are linked at a 100% similarity. However *E. coarctata* and *E. peltata* which belong to different sections are also linked at a 100% similarity.

DISCUSSION:

Character variation and pollination syndromes

The characters studied which could be influenced by selective pressures imposed by the specific vectors include exine sculpturing, tetrad shape and size.

Bird- pollinated species do not show any obvious form of aptation which could be related to the type of pollinator. The exine is not more heavily ornamented than species classified into either of the remaining pollination syndromes. Since the heavier ornamentation is related to the function of allowing for increase stickiness (Ferguson and Skvarla, 1982), it would be interesting to determine whether grains of bird-pollinated *Erica* species are in fact stickier

than their counterparts, despite the lack of heavy ornamentation. An independent experiment would need to be designed to test this since standard acetolysis procedures remove surface materials.

Comment cannot be made as to whether exines of bird-pollinated *Erica* species have an extra tectal layer, thus being more complex, as found in species of Papilionoideae (Ferguson and Skvarla, 1982). TEM would reveal stratification detail more clearly than LM used in this study. Heslop-Harrison (1986) did however point out that structural-functional relationships of exine stratification and sculpturing is complex and need not necessarily closely reflect obvious modification to the type of pollinator. This may possibly explain observations made in ornithophilous species of *Erica*.

Having found bird-pollinated species' tetrads to be statistically larger, would ~~invoke the conclusion~~ suggest that size is adapted to the type of pollinator. This would however not disprove that tetrads are larger merely because ornithophilous flowers are larger (allometric relationship). The fact that *E. hispidula*, a small wind dispersed species has larger tetrads than the larger flowered entomophilous species *E. leptopus* (Table 3) would tend to prove that tetrad size is independent of the type of vector. However this is also contrary to any allometric hypothesis. Lee

(cited by Simpson and Neff, 1983) states that flower size and pollen/tetrad size are generally correlated. More specifically pollen size is correlated to style length, implying that this correlation is of direct reproductive consequence. Any adaptation to the type of pollinator is thus secondary and therefore more an aptive than adaptive trait.

There is a trend in ornithophilous species to be clover shaped, and those species which are different vary only subtly. However the clover shape is not confined to ornithopilous species only, discounting this shape to be a selective consequence of the type of pollinator.

Where ornithopilous species show relative similarity in tetrad shape entomophilous species display the entire spectrum of recognized shapes. Shape may not be a character which would radically influence the efficiency of transport by biotic pollinators. Consequently there would not be any selective pressure to "mould" any particular shape. The uniformity in shape of ornithophilous species' tetrads therefore possibly reflects a closer phylogenetic relationship.

Not only do entomophilous species show large variation in shape, but in each of the characters studied the amount of variation makes the typification of an entomophilous tetrad

impossible. Ornamentation is also not as heavy as would be expected from observations made by Punt (1986).

A syndrome where selection does favour a particular shape is where pollen grains/tetrads need to be aerodynamically sound for wind transport (Crane, 1986). Globular or subglobular tetrads would therefore be expected. Many tetrads are clover to supraclover, the latter being found in *E. microcodon* and *E. rubiginosa*, which would question the correctness of their categorization as being anemophilous species. An alternative explanation to this phenomenon could be that the smaller size of tetrads, irrespective of being this particular shape, allows them to be windborne. A further aspect to consider is the fact that pollen grains are in a state of dehydration when transported (Heslop-Harrison, 1979; Crane, 1986). The harmomegathic systems in grains of abovementioned shapes may operate in a manner which alter the tetrad into a round, more aerodynamic, shape during dehydration (See Fig. 14e). This would imply that the harmomegathic function is directly involved in facilitating dispersal. The aerodynamics of tetrads of clover and supraclover shapes would make an interesting study.

The "ideal" psilate sculpturing type for wind-pollination is also not conformed to in anemophilous *Erica* species. The micro-type sculpturing (Traverse, 1988) which most of them display may however not render these tetrads less suitable

aerodynamically than a psilate surface. The micro-type sculpturing does not however distinguish anemophilous species form species in other syndromes since many micro-sculptured and psilate tetrads occur throughout.

The most anomelous results concerning adaptations/aptations of *Erica* pollen tetrad form as related to dispersal function are:

1. The many ornithophilous species with psilate and micro-type sculpturing.
2. The general lack of coarser sculpturing of biotically dispersed species.
3. The phenomenon of "apparently" unaerodynamic tetrad shapes in anemophilous species.
4. The lack of psilate surfaces in anemophilous species.
5. The lack of consistency in those character traits which may be influenced by the type of vector within each syndrome.

These findings would seem to support the statement by Simpson and Neff (1983) that no pollen characters have been conclusively demonstrated as having resulted from selection by pollinators, but are mostly explainable in terms of their reproductive functions. Particularly exine sculpturing patterns which are in the order of a few microns (< 2um in this study), would seem to be influenced little by pollen-

collecting structures of pollinating agents which are on the order of tenths of millimeters.

Pollen diversity and *Erica* sections

The absence of any pattern in tetrad characters within the majority of sections would suggest that on the basis of palynological data species within sections are not closely related. This suggestion would be supported by the results of the phenetic study as reflected in the dendrogram (Appendix 2). Although only a few characters were used in the analysis and mostly sections were represented by a few species, the trend is almost certainly a reflection of the situation within all sections. The fact that the sections and the species within sections were randomly selected would allow the assumption that should all species be tested, a similar result would probably be found.

Using palynological data would almost certainly disrupt the current classification into sections which is based on many floral morphological characters.

CONCLUSION:

The genus *Erica*, encompassing three pollination syndromes, presented the ideal taxon on which to test the hypothesis that the type of vector forms a selective pressure which alters pollen characters. These are then changed in a way which would maximally facilitate their transport. Results from this study do not prove this hypothesis, since no one character or set of characters can be used to delimit tetrads as belonging to any one particular pollination syndrome. The form of a tetrad and its constitutent grains would seem to indicate them being adapted primarily to the protection, harmomegethic and reservoir functions and secondary aptations as related to transport function are not sufficiently developed to allow distinct recognition. All variations would therefore appear to be due to their phylogeny and not the type of pollinator.

The absence of any trend within sections would seem to indicate that on the basis of palynological data, species within sections are polyphyletic. This conclusion is tentative as few sections within the study sample was represented by more than one species. These results would however tend to dispel the use of palynological characters as a taxonomic tool in any future reclassification attempt at lower taxonomic levels. Any future study would need to include TEM work to elucidate whether exine layers show

variations associated to type of pollinator or between subgenera or sections.

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REFERENCES:

BAKER, H.A. and OLIVER, E.G.H., 1967. Ericas in Southern Africa. Purnell, Cape Town.

BLACKMORE, S. and BARNES, Susan H., 1986. Harmomegathic mechanisms in pollen in pollen grains. In: S. Blackmore & I.K. Ferguson (eds). Pollen and Spores; Form and Function. Linnaen Society Symposium series No. 12. Academic Press, London. pp 137-150.

BENTHAM, G., 1876. Ericaceae. In: G. Bentham and J.D. Hooker, Genera Plantarum, 2: 577-604. Reeve, London.

CRANE, P.R., 1986. Form and function of wind dispersed pollen. In: S. Blackmore & I.K. Ferguson (eds). Pollen and Spores; Form and Function. Linnaen Society Symposium series No. 12. Academic Press, London. pp 179-202.

CRONQUIST, L., 1981. Integrated system of classification of flowering plants. The New Columbia University Trend, New York.

DRUDE, O., 1897. Ericaceae-Ericoideae. In: H.G.A. Engler and K.A.E. Prantl, Die Naturlichen Pflanzenfamilien, 4, 1: 57-65. Engelmann, Leipzig.

ERDTMAN, G., 1943. An introduction to pollen analysis. Chronica Botanica Company, Waltham Massachusets. pp 91-96.

ERDTMAN, G., 1971. Pollen morphology and plant taxonomy: Angiosperms - an introduction to palynology. Hafner Publishing Company, New York.

FERGUSON, I.K. & SKVARLA, J.J., 1982. Pollen morphology in relation to pollinators in Papilionoidae (Leguminosae). *Bot. J. Lin. Soc* 84: 183-193.

FOSS, P.J. & DOYLE, G.J., 1988. A palynological study of the Irish Ericaceae and *Empetrum*. *Pollen et spores* 30: 151-178.

HESLOP-HARRISON, J., 1979. Pollen walls as adaptive systems. *Ann. Missouri Bot. Gard.* 66: 813-819.

HESLOP-HARRISON, J., 1987. The adaptive significance of the exine. In: I.K. Ferguson and J. Muller (eds). The evolutionary significance of the exine, Linnaen Society Symposium Series No. 1. Henry King Ltd., Dorchester. pp 27-37.

KNOX, R.B. and McCONCHIE, C.A., 1986. Structure and function of compound pollen. In: Pollen and Spores; Form and Function, Linnaen Society Symposium Series No. 12. Blackmore, S. and Ferguson, I.K. (eds). Academic Press, London. pp 265-282.

LINDER, H.P. and FERGUSON, I.L., 1985. On the pollen morphology and phylogeny of the Restionales and Poales, *Grana,* 24: 65-76.

MULLER, J., 1979. Form and function in angiosperm pollen. *Ann. Missouri Bot. Gard.* 66: 594-632.

OLIVER, E.G.H., LINDER, H.P. and ROURKE, J.R., 1983. Geographical distribution of present day Cape taxa and their phytogeographical significance, *Bothalia,* 14: 427-440.

OLIVER, E.G.H., 1989. The Ericoidae and the Southern African heathers. *Bot. J. Lin. Soc.* 101: 319-327.

PUNT, W., 1984. Functional factors influencing pollen form. In: Pollen and Spores; Form and Function, Linnaen Society Symposium Series No. 12, Blackmore, S. and Ferguson, I.K. (eds), Academic Press, London. pp 97-101.

REBELO, A.G., SIEGFRIED, W.R. and OLIVER, E.G.H., 1985. Pollination syndromes of *Erica* species in the South Western Cape, *S. Afr. J. Bot,* 51(4): 270-280.

SIMPSON, Beryl B. and NEFF, J.L., 1983. Evolution and diversity of floral rewards. In: C.E. Jones and R.J. Little (eds). Handbook of pollination biology. pp 142-159.

STEVENS, P.F., 1971. A classification of the Ericaceae: subfamilies and tribes. *J. Linn. Soc.* (Botany), 64: 1-53.

STUESSY, T.F., 1990. Plant taxonomy: the systematic evaluation of conparative data. Columba University Press, New York. pp 267-287.

THAINIKAIMONI, G., 1986. Pollen apertures: form and function. In: S. Blackmore & I.K. Ferguson (eds). Pollen and Spores; Form and Function. Linnaen Society Symposium Series No. 12. Academic Press, London. pp 119-136.

TRAVERSE, A., 1988. Paleopalynology. Unwin Hyman Ltd., Boston. pp 60-116.

WALKER, J.W. and DOYLE, J.A., 1975. The bases of angiosperm phylogeny: Palynology, *Ann. Missouri Bot. Gard.* 62: 664-723.

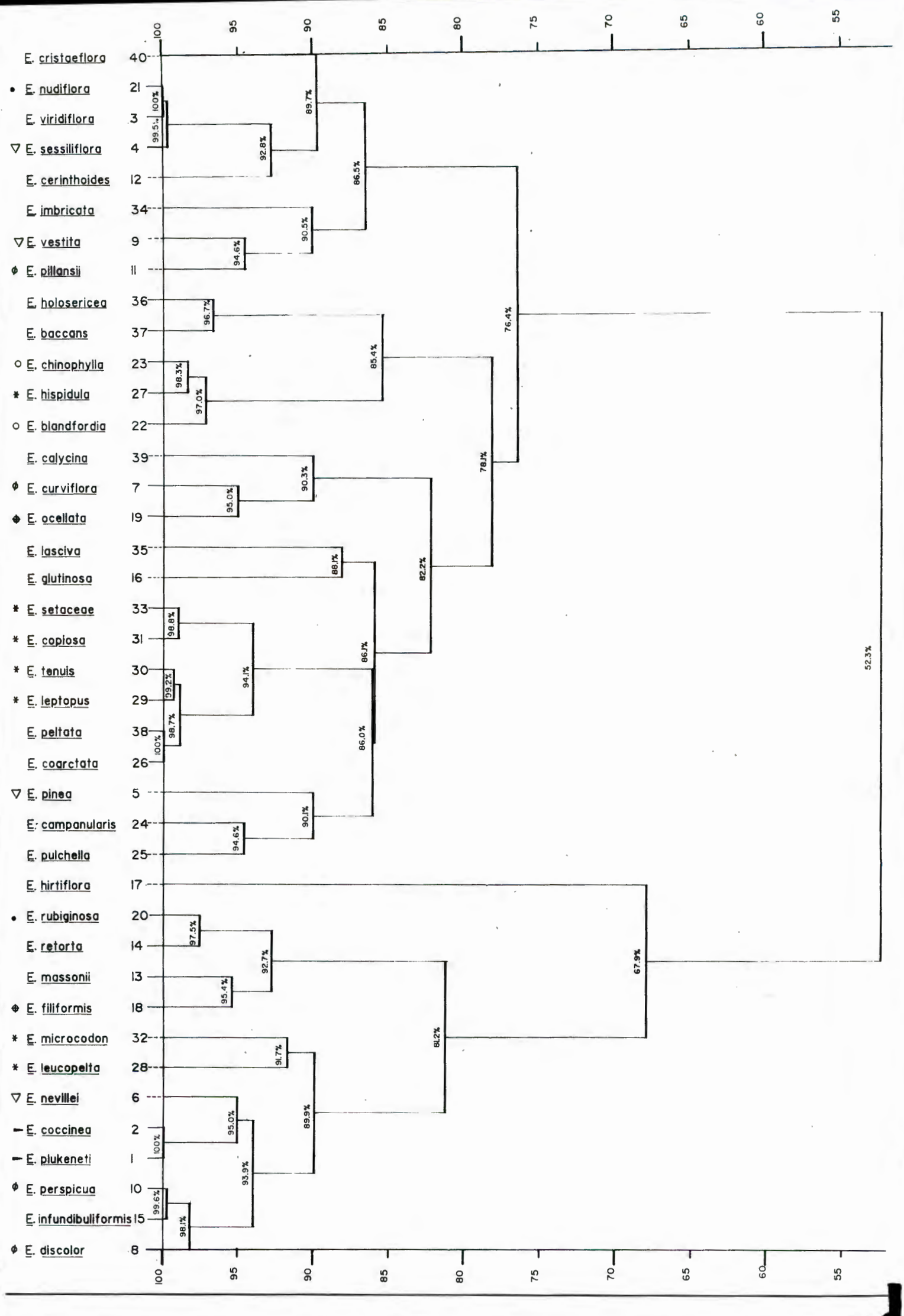
APPENDIX 1

List of *Erica* species used in this study. The name of the collector of the specimen from which the pollen sample was taken is shown. All pollen samples were taken from the Bolus Herbarium.

Taxa	Collector	Herbarium
E. plukeneti L.	Esterhuysen 1366	(BOL)
E. coccinea L.	Pillans 1058	(BOL)
E. viridiflora Andr.	Krige 13423	(BOL)
E. sessiliflora L.f.	Gusbrook (S.N.)	(BOL)
E. pinea Thunb.	Levyns 10472	(BOL)
E. nevillei L. Bol.	Leighton (S.N.)	(BOL)
E. curviflora L.	Pillans 8451	(BOL)
E. discolor Andr.	Lewis (S.N.)	(BOL)
E. vestita Thunb.	Leighton 1687	(BOL)
E. perspicua Wendl.	Levyns 11134	(BOL)
E. pillansii Bol.	Pillans 585	(BOL)
E. cerinthoides L.	Pillans 2524	(BOL)
E. massonii L.	Pillans 8314	(BOL)
E. retorta Montin	Pillans 8520	(BOL)
E. infundibuliformis Andr.	Esterhuysen 9582	(BOL)
E. glutinosa Berg.	Loubser 831	(BOL)
E. hirtiflora Curt.	Esterhuysen 10055	(BOL)
E. filiformis Salisb.	Esterhuysen 12615	(BOL)
E. ocellata Guth. & Bol.	Esterhuysen 33402	(BOL)
E. rubiginosa Dulfer	Esterhuysen 29616	(BOL)
E. nudiflora L.	Esterhuysen 15186	(BOL)
E. blandfordia Andr.	Oliver 30137	(BOL)
E. chionophylla Guth. & Bol.	Rose-Innis (S.N.)	(BOL)
E. campanularis Andr.	Levyns 3404	(BOL)
E. pulchella Houtt.	Esterhuysen 12615	(BOL)
E. coarctata Wendl.	Levyns 10851	(BOL)
E. hispidula L.	Esterhuysen 24485	(BOL)
E. leucopelta Tauch	Levyns 10661	(BOL)
E. leptopus Benth.	Esterhuysen 28120	(BOL)
E. tenuis Salisb.	Galpin 3713	(BOL)
E. copiosa Wendl.	Holland (S.N.)	(BOL)
E. microcodon Guth. & Bol.	Levyns 2472	(BOL)
E. setaceae Andr.	Compton 4060	(BOL)
E. imbricata L.	Leighton 1900	(BOL)
E. lasciva Salisb.	Esterhuysen 31948a	(BOL)
E. holosericea Salisb.	Esterhuysen 19167	(BOL)
E. baccans L.	Esterhuysen 11629	(BOL)
E. peltata Andr.	Esterhuysen 27188	(BOL)
E. calycina L.	Esterhuysen 21831	(BOL)
E. cristaeflora Salisb.	Esterhuysen 16140	(BOL)

APPENDIX 2

A dendrogram showing the grouping of *Erica* species according to the UNITER programme based on the characters, exine sculpturing, aperture margin type, tetrad width and tetrad shape. Linkages are shown as % similarity. Species with a similar asterisk shows they are from the same section.


100
95
90
85
80
75
70
65
60
55
E. cristaeflora 40
• E. nudiflora 21
E. viridiflora 3
▽ E. sessiliflora 4
E. cerinthoides 12
E. imbricata 34
▽ E. vestita 9
ϕ E. pillansii 11
E. holosericea 36
E. baccans 37
○ E. chinophylla 23
* E. hispidula 27
○ E. blandfordia 22
E. calycina 39
ϕ E. curviflora 7
⊕ E. ocellata 19
E. lasciva 35
E. glutinosa 16
* E. setaceae 33
* E. copiosa 31
* E. tenuis 30
* E. leptopus 29
E. peltata 38
E. cogrctata 26
▽ E. pinea 5
E. campanularis 24
E. pulchella 25
E. hirtiflora 17
• E. rubiginosa 20
E. retorta 14
E. massonii 13
⊕ E. filiformis 18
* E. microcodon 32
* E. leucopelta 28
▽ E. nevillei 6
— E. coccinea 2
— E. plukeneti 1
ϕ E. perspicua 10
E. infundibuliformis 15
ϕ E. discolor 8
99.5%
100%
89.7%
92.8%
86.5%
90.5%
94.6%
96.7%
76.4%
85.4%
98.3%
97.0%
78.1%
90.3%
95.0%
88.1%
82.2%
98.8%
86.1%
94.1%
99.2%
98.7%
100%
86.0%
90.1%
94.6%
52.3%
97.5%
92.7%
95.4%
67.9%
81.2%
91.7%
89.9%
95.0%
100%
93.9%
99.6%
98.1%